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(71) Applicant: ECC INTERNATIONAL INC. [US/US]; Suite 300, 100 Mansell Court East, Roswell, GA 30076 (US).

(72) Inventors: AJOKU, Kevin, I.; 203 Ruffed Grouse Drive, Imperial, PA 15126 (US). WIATR, Christopher, L.; 110 Scarborough Lane, McMurray, PA 15317 (US). MILLER, Patrick, C.; 1375 Royal Oak Drive, Wexford, PA 15020 (US).

(74) Agent: KIKEL, Suzanne; ECC International Inc., Calgon Center, 5400 Campbells Run Road, Pittsburgh, PA 15205 (US).

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(54) Title: SYNERGISTIC ANTIMICROBIAL COMPOSITION OF PEROXYACETIC ACID AND A PHOSPHORUS COMPOUND

(57) Abstract

Synergistic antimicrobial combinations are disclosed comprising an effective amount of peracetic acid (PAA) and an effective amount of a phosphorus compound selected from the group consisting of tetrakis (hydroxy methyl) phosphonium sulfate (THPS), tetrakis (hydroxy methyl) phosphonium phosphate (THPP), and tetrakis (hydroxy methyl) phosphonium chloride (THPC). Methods for inhibiting microbial growth using these synergistic antimicrobial combinations are also disclosed.

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TITLE OF THE INVENTION

SYNERGISTIC ANTIMICROBIAL COMPOSITION OF PEROXYACETIC ACID AND A PHOSPHORUS COMPOUND

FIELD OF THE INVENTION

The present invention relates to synergistic antimicrobial compositions which are generally useful for inhibiting microbial growth wherever such microbial growth is found, for example in aqueous systems related to a wide variety of industrial applications. More particularly, the present invention relates to synergistic admixtures of peroxyacetic acid and a phosphorus compound. Methods for using the same are also disclosed.

BACKGROUND OF THE INVENTION

Both peroxyacetic acid (sometimes referred to as peracetic acid) and certain phosphorus compounds such as tetrakis (hydroxy methyl) phosphonium sulfate, referred to herein as PAA and THPS respectively, are known individually as antimicrobial agents. The unexpected finding of the present invention is that they are synergistic when used in combination. As used herein, the terms "synergy" and "synergistic" refer to instances in which the effectiveness of a composition comprising two or more biocides, such as PAA and THPS, exceeds the sum of the efficacies of the individual components taken alone. Thus, using a synergistic biocidal combination may allow for use of a lower overall concentration of biocide or the realization of an enhanced antimicrobial effect at a comparable dosage.

Peroxyacetic acid, is known for its antimicrobial properties and its use as an antimicrobial agent is disclosed in U.S. Patent Nos. 5,368,749, 5,494,588, 5,624,575, 5,658,467, and 5,670,055. Disclosed is also synergistic mixtures of peroxyacetic acid and certain biocides as well as methods of using the same.

Likewise, the use of certain phosphorus compounds as antimicrobial agents, including tetrakis (hydroxy methyl) phosphonium sulfate (THPS) and related compounds is known and is disclosed in U.S. Patent Nos. 4,673,509 and 5,670,055. However, the

synergistic combination of PAA and THPS related compounds is not disclosed or suggested in the art.

As used herein, the phrases "antimicrobial", "biocide", and "inhibiting microbial growth" refer to the killing of, the inhibition of, or the control of the growth of bacteria, yeast, mold and/or algae. A number of important industries have experienced serious adverse effects from the activity of such biological growth on the raw materials which they employ, in their process waters, on various components of their manufacturing processes, and in the finished products which they produce. Such industries include the paint, wood, textile, cosmetic, leather, tobacco, fur, rope, paper, pulp, plastics, fuel, oil, rubber, and machine industries.

Systems which utilize circulating water or aqueous media become infected with microorganisms and experience substantial impairment of their efficiency when deposits of the microorganisms build up in the system. The deposits coat the walls of tanks and other vessels and any machinery or processing equipment which is employed and create blockages in pipes and valves. The deposits also create discolorations and other imperfections in the products being produced, forcing costly shutdowns. Control of microorganisms is particularly important in aqueous media in which there are dispersed particles or fines in the aqueous media, for example, dispersed cellulosic fibers and dispersed fillers and pigments in papermaking, and dispersed pigments in paint manufacture.

Slime control in papermaking processes is of particular importance. The control of bacteria and fungi in pulp and paper mill water systems which contain aqueous dispersions of papermaking fibers in various consistencies is especially critical. The uncontrolled buildup of slime produced by the accumulation of bacteria and fungi may cause off-grade production, decreased production due to down-time and greater cleanup frequency, increased raw material usage, and increased maintenance costs. The problem of slime deposits is especially critical in light of the widespread use of closed white water systems in the paper industry.

Another important area that requires the use of good antimicrobial compositions to control bacterial and fungal growth is in clay and pigment slurries. These slurries comprise various clays (e.g., kaolin) and pigments (e.g., calcium carbonate and titanium

dioxide) and usually are manufactured at a location separate from the end use application. This means that they are generally transported and stored for later use at the application site. Because of high quality standards for the paper and paint products in which such slurries are used, it is essential that these clay or pigment slurries have a very low microorganism count per gram of sample.

Accordingly, there remains a very real and substantial need for antimicrobial compositions capable of effectively controlling and/or inhibiting microbial growth in industrial aqueous systems and on articles of manufacture. Because of increasing environmental regulations, there is still a further need to provide biocidal compositions having enhanced antimicrobial effect which are effective in lower doses than historically used. Use of lower amounts of biocides has a favorable impact on the environment, and allows users to realize significant cost savings.

Additionally, there remains a substantial need for antimicrobial compositions that are, even at relatively high levels, safe for the environment.

SUMMARY OF THE INVENTION

The synergistic antimicrobial combination according to the present invention comprises an effective amount of peroxyacetic acid (PAA) and an effective amount of a phosphorus compound selected from the group consisting of tetrakis (hydroxy methyl) phosphonium sulfate (THPS), tetrakis (hydroxy methyl) phosphonium phosphate (THPP), and tetrakis (hydroxy methyl) phosphonium chloride (THPC). This amount of PAA and phosphorus compound can be any amount that provides an antimicrobial effect. Preferably this amount is an amount that, even if added in much higher amounts to the system, results in a synergy index (K value) of less than 1 after partial biocide degradation.

The synergistic antimicrobial combination according to the present invention can also comprise: a) PAA and b) a phosphorus compound selected from the group consisting of THPS, THPP, and THPC, wherein the weight ratio of a) to b), on an active basis, ranges between about 1000:1 and 1:1000.

The synergistic antimicrobial combination according to the present invention can also be in an aqueous concentrate that comprises: a) about 0.0001 to about 0.1 weight %

PAA; b) about 0.0001 to about 0.1 weight % of a phosphorus compound selected from the group consisting of THPS, THPP, and THPC; and c) the remainder being water (and other raw materials of the system being treated); wherein the weight ratio of a) to b), on an active basis, ranges between about 1000:1 and 1:1000.

The present invention also provides a method for inhibiting microbial growth in aqueous systems and on articles of manufacture prone to such growth comprising adding to said systems or applying to said articles an effective amount the synergistic antimicrobial combinations above.

DETAILED DESCRIPTION OF THE INVENTION

It has unexpectedly been discovered that the combination of PAA and the phosphorus compound (THPS, THPP, and/or THPC) is a synergistic antimicrobial combination, in that this combination generally meets the described antimicrobial needs for many applications. This synergistic combination is of even more value because of the desire in some industries to avoid the use of halogens to lessen the risk of adverse impact upon the environment.

As used herein, the term "effective amount" refers to that amount of a composition comprising PAA and the phosphorus compound necessary to achieve the desired level of inhibition or control of microbial growth in the aqueous system or on the article being treated.

The phosphorus compound used in the synergistic blend of the present invention is disclosed in U.S. Patent No. 4,673,509, the disclosure of which is incorporated herein by reference in its entirety. The phosphorus compound is preferably selected from the group consisting of THPS, THPP, and THPC, with THPS being most preferred. THPS is most preferred due to proven efficacy and availability, along with the fact that it is not a halogenated biocide.

It is contemplated that the synergistic admixture of PAA and the phosphorus compound, as disclosed herein, and the methods for using the same, will be useful in virtually any aqueous system or on any article or product of manufacture in which inhibition of microbial growth is desired, absent compatibility problems. Suggested applications of the synergistic antimicrobial combinations of the present invention

include, for example: inhibiting the growth of bacteria and fungi in aqueous paints, adhesives, latex emulsions, inks, joint cements and caulking compounds; preserving wood; preserving cutting oils and metal working fluids; controlling slime-producing bacteria and fungi, including yeast and mold, in pulp and paper mills and cooling towers; as a spray or dip treatment for textiles and leather to prevent mold growth; as a component of anti-fouling paints to prevent adherence of fouling organisms; protecting paint films, especially exterior paints, from attack by fungi which occurs during weathering of the paint film; protecting processing equipment from slime deposits during manufacture of cane and beet sugar, food, foodstuffs and food additives; preventing microorganism buildup and deposits in air washer or scrubber systems and in industrial fresh water supply systems; controlling microorganism contamination in closed loop and recirculating water cooling systems; controlling microorganism contamination and deposits in oil field drilling fluids and muds, and in secondary petroleum recovery processes; preventing bacterial and fungal growth in paper coating processes which might adversely affect the quality of the paper coating; controlling bacterial and fungal growth and deposits during the manufacture of various specialty boards, e.g., cardboard, particle board and food grade board; preventing sap stain discoloration on freshly cut wood of various kinds; controlling bacterial and fungal growth in clay and pigment slurries of various types which are manufactured for later use in paper coating and paint manufacturing and which are susceptible to degradation by microorganisms during storage and transport; as a hard surface disinfectant to prevent growth of bacteria and fungi on walls, floors, etc.; in swimming pools to prevent algal growth, including green algae and cyanobacteria (blue green algae); and to control bacterial and fungal growth in various cosmetic products. It is further contemplated that the synergistic admixture of the present invention will be useful in various types of non-aqueous systems as well.

The synergistic antimicrobial composition disclosed in the present invention is particularly applicable to microbial slime control in papermaking processes. The need to control bacteria and fungi in pulp and paper mill water systems which contain aqueous dispersions of papermaking fibers in various consistencies is disclosed above.

Another important area in which the antimicrobial compositions of the present invention are particularly useful is in the control of bacterial and fungal growth in clay

and pigment slurries, as disclosed above. It is essential that these clay or pigment slurries have a very low microorganism count per gram sample.

In addition, the synergistic combination of the present invention and methods of using the same have been found especially useful in controlling the harmful effects of microorganisms in water or aqueous media. Particular examples of aqueous systems that have a need for the use of the present invention to control microorganisms include cooling waters used in the chemical industry and in power generation, wastewater treatment from municipal and industrial plants, as well as pasteurization systems in the food and beverage industry.

As disclosed above, any system which utilizes circulating water or aqueous media becomes infected with microorganisms and experience substantial impairment of their efficiency when deposits of the microorganisms build up in the system and can be treated according to the present invention. Control of microorganisms by the present invention in aqueous media is particularly important where there are dispersed particles or fines in the aqueous media, for example, dispersed cellulosic fibers and dispersed fillers and pigments in papermaking, and dispersed pigments in paint manufacture.

The present invention is directed to a synergistic antimicrobial composition comprising: a) PAA; and b) a phosphorus compound selected from the group consisting of THPS, THPP, and THPC, wherein the weight ratio of a) to b), on an active basis, preferably ranges from about 1000:1 to 1:1000. The present invention is further directed to a method for inhibiting microbial growth in an aqueous system or on an article of manufacture prone to such growth, which method comprises treating said system or said article with an effective amount of an antimicrobial composition comprising: a) PAA; and b) a phosphorus compound selected from the group consisting of THPS, THPP, and THPC, wherein the weight ratio of a) to b), on an active basis, ranges from about 1000:1 to 1:1000.

In accordance with the present invention, the weight ratios of the two components of the synergistic combination are dictated by the dosage levels of each component which demonstrate synergism, based on 100% active ingredient, relative to each end use application. Typically, the weight ratio of component a), PAA, and component b), the phosphorus compound, ranges from about 1000:1 to 1:1000 on an active basis, preferably

from about 100:1 to 1:100, more preferably from about 10:1 to 1:10. As will be understood by one skilled in the art, however, the synergistic weight ratio of the two components generally varies to some extent depending on the application and the organism being controlled. For example, a higher ratio of PAA to the phosphorus compound might be more effective in one application, while a higher ratio of the phosphorus compound to PAA might be more effective in another application. The PAA/phosphorus compound composition has been found particularly effective against bacteria when used in a weight ratio of between about 5:1 to 1:5.

In the aqueous system being treated with the synergistic composition of PAA and the phosphorus compound, the amount of each biocide present can range from about 0.05 parts per million (ppm) to about 200 ppm. Preferably, between about 1 ppm and about 100 ppm of each biocide based on the weight of water in the system being treated, are added, more preferably, between about 2 ppm and 60 ppm. However, an effective amount of the synergistic combination of the present invention must be added to the aqueous system being treated even if there is only a very small amount of one biocide and a larger amount of the other. This amount of the synergistic composition of the present invention should at least be about 0.1 ppm based on the weight of water in the system being treated, preferably at least 1 ppm, more preferably at least 10 ppm.

The upper limits of each component and the upper limits of the total synergistic combination of the present invention depends upon economics and environmental concerns. The total amount of the inventive synergistic biocide combination present in an aqueous system should be less that about 300 ppm, preferably less that about 200 ppm, more preferably no more than about 150 ppm, with an amount no more than about 100 ppm being most preferred. However, it is well within the ordinary skill of one practicing in the art to determine the effective amount of biocide for a given system based on various system parameters including but not limited to the size of the system, pH of the system, the types of organisms present and the amount of control desired.

Additionally, in some applications a higher overall percent biocide is required to control or kill the microbes. In these applications, higher amounts of each biocide can be added while maintaining a measurable synergistic effect. Thus, the upper limit of the synergy of the combination varies, depending on the application. In preservation

applications for example, each biocide in the blend could be present in a concentration ranging from about 0.05 ppm up to as high as 1000 ppm.

Much higher amounts of one or both biocides can be added to the system to provide protection from microbial growth since the concentration throughout the entire system will not necessarily be the same and the localized ratios can very widely. Also, initial higher amounts can be added with the anticipation that one or both biocides will degrade or decompose over time to then be within the ranges stated above. Even though higher amounts than stated above are added to the system to be treated, what is important is that the localized amounts or time degraded amounts fall within the stated range at some point in time, thereby benefiting from the synergistic combination as the microbes in the system are controlled.

Additionally, when one of the two biocides in the present biocide blend is added at a relatively high concentration (e.g. 200 ppm or more) there still is a synergistic effect at the addition of the second biocide but that effect is masked by the overall high concentration of the total biocide present in the system. For example, if the phosphorus compound THPS is added in and amount greater than 200 ppm to a system containing E.coli at the same time an amount of PAA is added, the kill rate would be faster than with THPS alone. However, the final kill or inhibition effect would not be significantly different because all of the E.coli bacteria are eventually killed with 200 ppm of THPS all by itself.

An effective amount of a synergistic combination of PAA and the phosphorus compound can also be applied to the article of manufacture being treated. Generally, a solution of the synergistic antimicrobial combination described above having a concentration of at least 0.1 ppm is incorporated into, sprayed or poured onto, used to dip, or otherwise applied, for example by dipping or submersing, to the substrate being treated in order to prevent growth of bacteria, mold, yeast and algae. Again, it is well within the ordinary skill of one practicing in the art to determine the effective amount of biocide to apply to a given article of manufacture being treated and to determine suitable modes of application.

The active ingredients of the synergistic antimicrobial compositions of the present invention may also be used in diverse formulations: solid, including finely divided

powders and granular materials; as well as liquid, such as solutions, emulsions, suspensions, concentrates, emulsifiable concentrates, slurries and the like, depending upon the application intended, and the formulation media desired. Further, when the synergistic antimicrobial combinations are liquid, they may be employed neat or may be incorporated into various formulations, both solid and liquid, as an adsorbate on suitable inert carriers such as talc, clays, diatomaceous earth and the like, or water and various organic liquids such as lower alkanols, kerosene, benzene, toluene, and other petroleum distillate fractions or mixtures thereof.

The amount of the synergistic combination of PAA and the phosphorus compound can be added to the aqueous system being treated as solid single components or as a dry blend. However, the synergistic combination is preferably added as a solution that is an aqueous concentration to be diluted *in situ* to the above amounts. This synergistic combination is preferably added as two separate single component solutions to be blended and diluted *in situ*. This separate addition is preferred due to the instability of blend solution over time due to degradation. PAA is an oxidizing biocide and the phosphorus compound is a non-oxidizing biocide. Thus, over time PAA degrades in the solution blend as it degrades and oxidizes the phosphorus compound (THPS, THPP, and/or THPC).

The aqueous concentrate of each of the single component solutions used in the synergistic antimicrobial combination would contain much higher amounts of each of the biocides than would be present in the final treated aqueous system and is well within the ordinary skill of one practicing in the art . This concentrate can vary widely depending upon the weight savings desired with reduced amounts of water while maintaining ease of application with minimal mixing in situ. What is important is the ratio and final concentration of each of the biocides in the treated aqueous system. These should be within the limits stated above. Each concentrate can contain other standard components and be added in combination with other standard additives used in the particular aqueous system.

PAA is commercially available in liquid form from English China Clays Inc./Calgon Corporation (ECC/Calgon), Pittsburgh, PA as Metasol® PAA, which is 12% active PAA, CH_3COOOH. This product also contains about 18% hydrogen peroxide,

about 20% acetic acid, and about 50% water. THPS is also commercially available from ECC/Calgon, Pittsburgh, PA and is in liquid form as Metasol® LT, which is 35% active THPS in a water solution.

To prepare the two solutions of the synergistic composition under this invention, an effective amount of each active ingredient should be combined in a suitable carrier such as water, organic solvents and the like. The preparation of such a composition is within the ordinary skill of one practicing in the art.

It will also be understood by one skilled in the art that the synergistic antimicrobial combination disclosed herein may be used in combination with other antimicrobial materials. For example, the combination can be combined with other fungicides and bactericides in appropriate concentrations and in appropriate instances so as to combine the action of each to obtain particularly useful results. Such combinations might find particular application in the preparation of germicidal soaps, in the production of cosmetics and aqueous coatings and in combating paper mill microbial slime accumulations. It is clear also that the synergistic antimicrobial combination of the present invention can be combined with other algicidal agents as well.

In accordance with the present invention there is still further provided a method of inhibiting the growth of at least one of the following: bacteria, yeast, mold and algae. According to the methods of the present invention, this growth is inhibited in aqueous systems or on articles or products of manufacture prone to such growth. These methods comprise adding to the aqueous system or treating the article or product containing said bacteria, yeast, mold and/or algae with an effective amount of a synergistic combination of PAA and the phosphorus compound (e.g. THPS). This addition can be accomplished either by simple addition of components together as a single admixture, or by the preferred addition of the two components separately. Such separate administration can either be at the same time or at different times. The net effect will be the same--the system, article or product being treated will ultimately have incorporated therein or have applied thereto the desired dosage concentration of each component.

Further, the compositions of the present invention are believed to be effective irrespective of the method of application (unless the blend is stored for a period of time prior to addition). For example, the antimicrobial compositions described herein can be

added to a system being treated as two separate or one stream via a low level, continuous feed practice, a semi-continuous feed practice or through slug feeding. All of these feeding practices will be familiar to one having ordinary skill in the art. Slug feeding is particularly effective and therefore is a preferred manner of employing the methods of the present invention. This type of feed allows the user to monitor the microorganism concentration in the system and feed product only when microorganism concentrations increase. The user realizes a cost savings by feeding an effective amount of PAA and the phosphorus compound only when needed.

As noted above, the present invention is based upon the discovery that use of PAA in conjunction with certain types of phosphorus compounds produces synergistic results and is effective in controlling the growth of bacteria, yeast, mold and algae in a variety of industrial and other applications. The utility of the synergistic antimicrobial combination disclosed herein derives from its versatility both in the numerous industries in which it can be applied, as well as the numerous microorganisms against which it is effective. In particular, the large economic losses in papermaking operations caused by the accumulation of bacterial and fungal slimes in various parts of the system can be eliminated to a significant extent by use of the synergistic combination described herein.

The superior antimicrobial activity of the synergistic antimicrobial combination of PAA and the phosphorus compound has been confirmed using standard laboratory techniques. The antimicrobial combination has been found effective, for example, in inhibiting bacterial growth including but not limited to *Klebsiella pneumoniae* and *Escherichia coli* and has been found to be particularly effective against *Pseudomonas aeruginosa*. The combination is also believed to be effective against other aerobic bacteria, such as *Bacillus*, *Staphylococcus*, *Flavobacterium*, *Enterobacter*, and *Xanthomonas*, anaerobic bacteria, freshwater organisms such as filamentous bacteria, fungi including but not limited to various species of *Candida* and *Saccharomyces*, white and pink yeasts, molds, and various species of green algae and blue green algae.

EXAMPLES

The following examples are set forth to illustrate the present invention and should not be construed as limiting the invention in any way.

EXAMPLE I

The biocidal efficacy in microtiter tests of the antimicrobial composition of the present invention is demonstrated below. Three different bacterial strains, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, as well as a mixture of all three of the strains, were used.

Each of the three bacteria were separately grown on Standard Methods Agar (STM) plates and incubated at 37°C for a period of between about 24-48 hours. Each species of bacteria was inoculated into a small tissue flask containing approximately 25 ml of Allen's Media using a sterile swab. The cultures were then diluted by adding 10 ml of bacterial suspension to 90 ml of fresh Allen's Media. To prepare a mixed culture of all three of the organisms, an equal amount of approximately 20 ml, of each of the diluted cultures was mixed together in a large tissue culture flask. Samples from the mixture and the individual bacterial suspensions were then used in the microtiter test.

An 8X stock solution of PAA to use in combination with THPS was prepared by dissolving about 6.6 grams (g) of 12% active PAA in about 100 ml of deionized water. A 4X stock solution of THPS to use in combination with PAA was prepared in the same manner only using 0.106 g of THPS (35% active THPS). A 4X stock solution of THPS to use alone was prepared in the same manner only using 0.53 g of THPS. A PAA 8X stock solution to use in combination with THPS was prepared by dissolving about 6.6 g of 12% active PAA in 100 ml with deionized water. A 4X stock solution of THPS to use in combination with PAA was prepared in the same manner only using 0.05 g THPS. The 4x stock solution of PAA to use alone was prepared in the same manner only using 3.3 g PAA. Subsequently, two other plates were made up using 6.6 grams of PAA and 0.16 g and 0.26 g THPS respectively. Eight microtiter plates were used in the example (not counting the plates for PAA and THPS alone), each microtiter plate having 8 rows, A-H, and 12 columns, 1-12. The amount of each biocide in each well of the eight plates is depicted below.

TABLE I

AMOUNT OF EACH BIOCIDE IN WELLS OF Microtiter PLATES 1-8

PLATE #	BIOCIDE	COLUMN CONCENTRATIONS (ppm Active)											
		1	2	3	4	5	6	7	8	9	10	11	12
1,5	PAA (8X)	1000	500	250	125	62	31	16	8	4	2	-	+
	THPS (4X)	200	200	200	200	200	200	200	200	200	200	-	+
2,6	PAA (8X)	1000	500	250	125	62	31	16	8	4	2	-	+
	THPS (4X)	100	100	100	100	100	100	100	100	100	100	-	+
3,7	PAA (8X)	1000	500	250	125	62	31	16	8	4	2	-	+
	THPS (4X)	300	300	300	300	300	300	300	300	300	300	-	+
4,8	PAA (8X)	1000	500	250	125	62	31	16	8	4	2	-	+
	THPS (4X)	500	500	500	500	500	500	500	500	500	500	-	+
9	PAA (8X)	1000	500	250	125	62	31	16	8	4	2	-	+
	THPS (4X)	0	0	0	0	0	0	0	0	0	0	-	+
10	PAA (8X)	0	0	0	0	0	0	0	0	0	0	-	+
	THPS (4X)	1000	500	250	125	62	31	16	8	4	2	-	+

As is illustrated in the table above, the amount of PAA in the wells of plates 1 through 9 were varied in a serial dilution series ranging from 1000 ppm active to 2 ppm active while the other component's (THPS) concentration was kept constant at 200, 100, 300, 500, and 0 ppm active. Plate 10 represented use of THPS alone in a serial dilution series. Plates 9 and 10 represented the use of each biocide alone and were used to determine the minimum amount of each biocide which, when used alone, would inhibit microbial growth. No biocide was added to the wells of column 12 in any of the plates, which represented an organism control, or positive control. This positive control was run to ensure that the organisms were capable of growing in the environment provided. No bacteria were added to the wells of column 11 in any of the plates, which represented the Allen's Media control, or a negative control. This was done to ensure that there was no contamination of the plates. In each of the 10 microtiter plates *Pseudomonas aeruginosa*

was added to rows A and B, *Klebsiella pneumoniae* to rows C and D, *Escherichia coli* to rows E and F, and the mix of all three bacteria to rows G and H.

Plates 1-4 were used to determine the minimum inhibitory concentration (MIC) for each biocide combination against each bacteria strain. The MIC is the least amount of biocide needed to prevent growth in the well, with growth being defined as a turbidity in the medium or a "pellet" of cells which came out of the medium and settled at the bottom of the well.

Plates 5-8 were then subcultured from plates 1-4, respectively, at 24 hours following biocide addition. Subculturing was done to determine the minimum biocidal concentration (MBC). The MBC is the lowest concentration of biocide that results in no growth after subculturing and subsequent incubation.

All of the microtiter plates including the MIC plates and the MBC plates were incubated for 24 hours at 37°C. Following the 24 hour incubation period, the presence or absence of growth in each well of the plates was determined. Growth in the microtiter plates was determined by subculturing the plates onto a solid agar plate dipicting each microtiter well. The plates were then incubated at 37°C for 24 hours. The presence or absence of growth in each well, along with the concentration of biocide in each well, was then used to determine the synergistic properties of the biocide combinations. The synergistic properties were evaluated by determining the Kull value/K value; the K value was determined for each bacterium tested. The method for calculating K value is well known to those skilled in the art. In this example, the K value was determined by the following formula:

$$K = \frac{[\text{PAA}]\ \text{In Combination}}{[\text{PAA}]\ \text{Alone}} + \frac{[\text{THPS}]\ \text{In Combination}}{[\text{THPS}]\ \text{Alone}}$$

where "[PAA] In Combination" means the concentration of PAA which, when used in combination with THPS, resulted in inhibition of microbial growth;

"[THPS] In Combination" means the concentration of THPS which, when used in combination with PAA, resulted in inhibition of microbial growth;

"[PAA] Alone" means the concentration of the PAA which, when used alone, resulted in inhibition of microbial growth; and

"[THPS] Alone" means the concentration of THPS which, when used alone, resulted in inhibition of microbial growth.

A K value of less than 1 indicates synergy between the two biocides, a K value of greater than 1 indicates antagonism between the two biocides, and a K value equal to 1 indicates an additive effect of the two biocides.

The K values determined for each of the organisms used in the example are recorded in Tables 2 through 9.

TABLE 2

"K" VALUES OF PLATE 1 (MIC)

Organism	[PAA] Alone, ppm	[THPS Alone, ppm	[PAA] In Combination, ppm	[THPS] In Combination, ppm	K Value	Weight Ratio PAA: THPS
Pseudomonas aeruginosa	1000	125	3.9	200	1.6	0.0195:1
Klebsiella pneumoniae	93.75	125	5.85	200	1.7	0.0293:1
Escherichia coli	7.8	125	1.95	200	1.9	0.0098:1
Mixture of above three	125	125	5.85	200	1.6	0.0293:1

TABLE 3

"K" VALUES OF PLATE 2 (MIC)

Organism	[PAA] Alone, ppm	[THPS] Alone, ppm	[PAA] In Combination, ppm	[THPS] In Combination ppm	K Value	Weight Ratio PAA: THPS
Psuedomonas aeruginosa	1000	125	5.8	100	0.81	0.058:1
Klebsiella pneumoniae	93.75	125	3.9	100	0.84	0.04:1
Escherichia coli	7.8	125	1.95	100	1.05	0.019:1
Mixture of above three	125	125	23.4	100	0.99	0.234:1

TABLE 4

"K" VALUES OF PLATE 3 (MIC)

Organism	[PAA] Alone, ppm	[THPS] Alone, ppm	[PAA] In Combination, ppm	[THPS] In Combination ppm	K Value	Weight Ratio PAA: THPS
Pseudomonas aeruginosa	1000	125	15.6	300	2.42	0.052:1
Klebsiella pneumoniae	93.75	125	31.2	300	2.73	0.104:1
Escherichia coli	7.8	125	1.95	300	2.65	0.007:1
Mixture of above three	125	125	15.6	300	2.52	0.052:1

TABLE 5

"K" VALUES OF PLATE 4(MIC)

Organism	[PAA] Alone, ppm	[THPS] Alone, ppm	[PAA] In Combination, ppm	[THPS] In Combination ppm	K Value	Weight Ratio PAA: THPS
Pseudomonas aeruginosa	1000	125	3.9	500	4.00	0.008:1
Klebsiella pneumoniae	93.75	125	5.85	500	4.06	0.012:1
Escherichia coli	7.8	125	1.95	500	4.25	0.004:1
Mixture of above three	125	125	5.85	500	4.05	0.012:1

TABLE 6

"K" VALUES OF PLATE 5 (MBC)

Organism	[PAA] Alone, ppm	[THPS] Alone, ppm	[PAA] In Combination, ppm	[THPS] In Combination ppm	K Value	Weight Ratio PAA: THPS
Pseudomonas aeruginosa	62.5	125	3.9	200	1.66	0.0195:1
Klebsiella pneumoniae	78.1	125	5.85	200	1.67	0.029:1
Escherichia coli	15.6	125	1.95	200	1.73	0.009:1
Mixture of above three	125	125	5.85	200	1.65	0.029:1

TABLE 7

"K" VALUES OF PLATE 6 (MBC)

Organism	[PAA] Alone, ppm	[THPS] Alone, ppm	[PAA] In Combination, ppm	[THPS] In Combination ppm	K Value	Weight Ratio PAA: THPS
Pseudomonas aeruginosa	62.5	125	2.9	100	0.85	0.029:1
Klebsiella pneumoniae	78.1	125	3.9	100	0.85	0.04:1
Escherichia coli	15.6	125	1.95	100	0.93	0.019:1
Mixture of above three	125	125	3.9	100	0.83	0.04:1

TABLE 8

"K" VALUES OF PLATE 7 (MBC)

Organism	[PAA] Alone, ppm	[THPS] Alone, ppm	[PAA] In Combination, ppm	[THPS] In Combination ppm	K Value	Weight Ratio PAA: THPS
Pseudomonas aeruginosa	62.5	125	15.6	300	2.65	0.052:1
Klebsiella pneumoniae	78.1	125	31.9	300	2.80	0.1:1
Escherichia coli	15.6	125	1.95	300	2.53	0.006:1
Mixture of above three	125	125	15.6	300	2.52	0.052:1

TABLE 9

"K" VALUES OF PLATE 8 (MBC)

Organism	[PAA] Alone, ppm	[THPS] Alone, ppm	[PAA] In Combination, ppm	[THPS] In Combination ppm	K Value	Weight Ratio PAA: THPS
Pseudomonas aeruginosa	62.5	125	2.9	500	4.05	0.006:1
Klebsiella pneumoniae	78.1	125	5.85	500	4.07	0.012:1
Escherichia coli	15.6	125	1.95	500	4.13	0.004:1
Mixture of above three	125	125	7.8	500	4.06	0.015:1

WHAT IS CLAIMED IS:

1. An antimicrobial combination comprising an effective amount of peracetic acid (PAA) and an effective amount of a phosphorus compound selected from the group consisting of tetrakis (hydroxy methyl) phosphonium sulfate (THPS), tetrakis (hydroxy methyl) phosphonium phosphate (THPP), and tetrakis (hydroxy methyl) phosphonium chloride (THPC).

2. The antimicrobial combination composition according to claim 1 wherein said amount of peracetic acid and phosphorus compound is an amount that results in a synergy index (K value) of less than 1, determined by the following formula in which THPS represents the phosphorus compound:

$$K = \frac{\text{(PAA) In Combination}}{\text{(PAA) Alone}} + \frac{\text{(THPS) In Combination}}{\text{(THPS) Alone}}$$

wherein "(PAA) In Combination" means the concentration of PAA which, when used in combination with THPS, resulted in inhibition of microbial growth; "(THPS) In Combination" means the concentration of THPS which, when used in combination with PAA, resulted in inhibition of microbial growth; "(PAA) Alone" means the concentration of the PAA which, when used alone, resulted in inhibition of microbial growth; and "(THPS) Alone" means the concentration of THPS which, when used alone, resulted in inhibition of microbial growth.

3. The antimicrobial combination composition according to claim 1 wherein a) PAA and b) the phosphorus compound, THPS, THPP, or THPC, are present in an aqueous system at a weight ratio of a) to b), on an active basis, between about 1000:1 and 1:1000.

4. The antimicrobial combination composition according to claim 1 wherein the combination is in an aqueous concentrate that comprises: a) about 0.0001 to about 0.1 weight % PAA; b) about 0.0001 to about 0.1 weight % of the phosphorus compound; and c) the remainder being water; wherein the weight ratio of a) to b), on an active basis, ranges between about 1000:1 and 1:1000.

5. The antimicrobial combination composition according to claim 1 wherein the combination is first in two separate aqueous concentrates that comprises: a) about 0.0001 to about 0.1 weight % PAA; and b) about 0.0001 to about 0.1 weight % of the phosphorus compound with the remainder of each concentrate being water; wherein the weight ratio of a) to b) on an active basis once combined, ranges between about 1000:1 and 1:1000.

6. A synergistic antimicrobial combination comprising:

a) peracetic acid and

b) a phosphorus compound selected from the group consisting of THPS, THPP, and THPC;

wherein the weight ratio of a) to b), on an active basis, ranges between about 1000:1 and 1:1000.

7. The composition according to Claim 6 wherein the phosphorus compound is THPS and the weight ratio of a) to b) ranges between about 100:1 and 1:100.

8. A method for inhibiting microbial growth in an aqueous system which comprises adding to said system an effective amount of the synergistic antimicrobial combination of claim 1.

9. A method for inhibiting microbial growth in an aqueous system which comprises adding to said system an effective amount of the synergistic antimicrobial combination of claim 6.

10. The method according to Claim 9 wherein the weight ratio of a) to b) ranges between about 10:1 and 1:10.

11. The method according to claim 9 wherein the peracetic acid and the phosphorus compound are added together as a single composition to the system being treated.

12. The method according to Claim 9 wherein the peracetic acid and the phosphorus compound are added separately to the system being treated.

13. The method according to Claim 9 wherein at least 0.1 ppm of the synergistic antimicrobial composition is added to a system selected from the group consisting of; paper mill water systems, clay and pigment slurry systems, cooling water systems, and wastewater treatment systems.

14. The method according to Claim 9 wherein about 1 ppm to about 100 ppm peracetic acid and about 1 ppm to about 100 ppm of the phosphorus compound THPS are added to the system being treated.

15. A method for inhibiting microbial growth on an article of manufacture which comprises applying to said article an effective amount of the synergistic antimicrobial combination of claim 1.

16. A method for inhibiting microbial growth on an article of manufacture which comprises applying to said article an effective amount of the antimicrobial combination composition of claim 6.

17. The method according to Claim 16 wherein the weight ratio of a) to b) ranges between about 100:1 and 1:100.

18. The method according to claim 16 wherein the peracetic acid and the phosphorus compound are added together as a single composition to the article being treated.

19. The method according to Claim 16 wherein the peracetic acid and the phosphorus compound are added separately to the article being treated.

20. The method according to Claim 16 wherein said synergistic antimicrobial composition is in a concentration of at least 0.1 ppm.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/16463

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A01N57/34 //(A01N57/34,37:16)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 14092 A (GRACE W R & CO) 17 May 1996 (1996-05-17) claims	1-20

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 October 1999	03/11/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Decorte, D

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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PCT/US 99/16463

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